When plants are grown in elevated carbon dioxide concentrations, which genes control the stimulation of metabolism and growth?

Ryan Boyd
Andrew Leakey

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Introduction:
Plants and CO_2

- The release of CO_2 as a result of respiration in plants is a major component of the global carbon cycle (Canadell *et al.* 2007). A better understanding of carbon cycling is needed if a thorough understanding of climate change, let alone a successful technical solution to climate change is to be implemented. The rise in atmospheric [CO_2] has already been shown to stimulate photosynthesis in plants; but this stimulation in photosynthesis is useless to the plant if it cannot utilize the increased sugars produced by the increased rate of photosynthesis (Ainsworth, Rogers & Leakey 2007). How then does respiration in plants grown at elevated [CO_2] respond to utilize the increased sugar production?

JG Canadell, C Le Quere , MR Raupach, CB Field, ET Buitenhuis, P Ciais, TJ Conway, NP Gillett, RA Houghton & G Marland (2007) Contributions to accelerating atmospheric CO2 growth from economic activity, carbon intensity, and efficiency of natural sinks. *Proceedings of the National Academy of Sciences* 104: 18866-18870.

EA Ainsworth, A Rogers, ADB Leakey (2008) Targets for crop biotechnology in a future high-CO2 and high-O3 world. *Plant Physiology* 147:13-19.

Introduction:

CO_2 and Gene Regulation

Studies at the SoyFACE (Soybean Free Air Concentration Enrichment) field-research facility have revealed that nighttime, foliar respiration of soybeans grown under elevated [CO2] is stimulated by 39% compared to soybeans grown under ambient [CO2] (Leakey et al. 2009). This coincided with greater gene expression for many enzymes involved in glycolysis, the tricarboxylic acid cycle and mitochondrial electron transport. This suggests that changes in gene expression may explain the greater numbers of mitochondria per cell that have been observed in leaves of many plant species when they are grown at elevated [CO2] (Griffin et al. 2001), and in turn greater rates of respiration. Twenty five of the transcripts that were up-regulated in soybean grown at elevated [CO2] encode transcription factors.

The question this project addresses is: Do any of the transcription factors that were observed to be up-regulated by growth at elevated [CO2] in soybean act to control the observed increase in respiration rates and growth?

ADB Leakey, F Xu, K Gillespie, JM McGrath, EA Ainsworth & DR Ort. (2009) Genomic basis for stimulated respiratory carbon loss to the atmosphere by plants growing under elevated CO2. *Proceedings of the National Academy of Sciences.* 106: 3597-3602.

KL Griffin, OR Anderson, MD Gastrich, JD Lewis, G Lin, W Schuster, JR Seeman, DT Tissue, MH Turnbull & D Whitehead (2001) Plant growth in elevated CO2 alters mitochondrial number and chloroplast fine structure. *Proceedings of the National Academy of Sciences* 98: 2473-2478.

Introduction:

Importance of Transcription Factors

- Transcription factors are important because of their ability to regulate the transcription of other genes.
- Because of this ability a transcription factor should have a larger effect than a single gene.
- This is why we chose to work with the 25 transcription factors during this screening process.

Approach

- Genetic screening allows us a preliminary look at a gene’s function without in depth research and experimentation.
- Using mutants we can observe any phenotypes different from the wild type. This allows us to ask the question: When this gene is not functioning what happens?

Approach

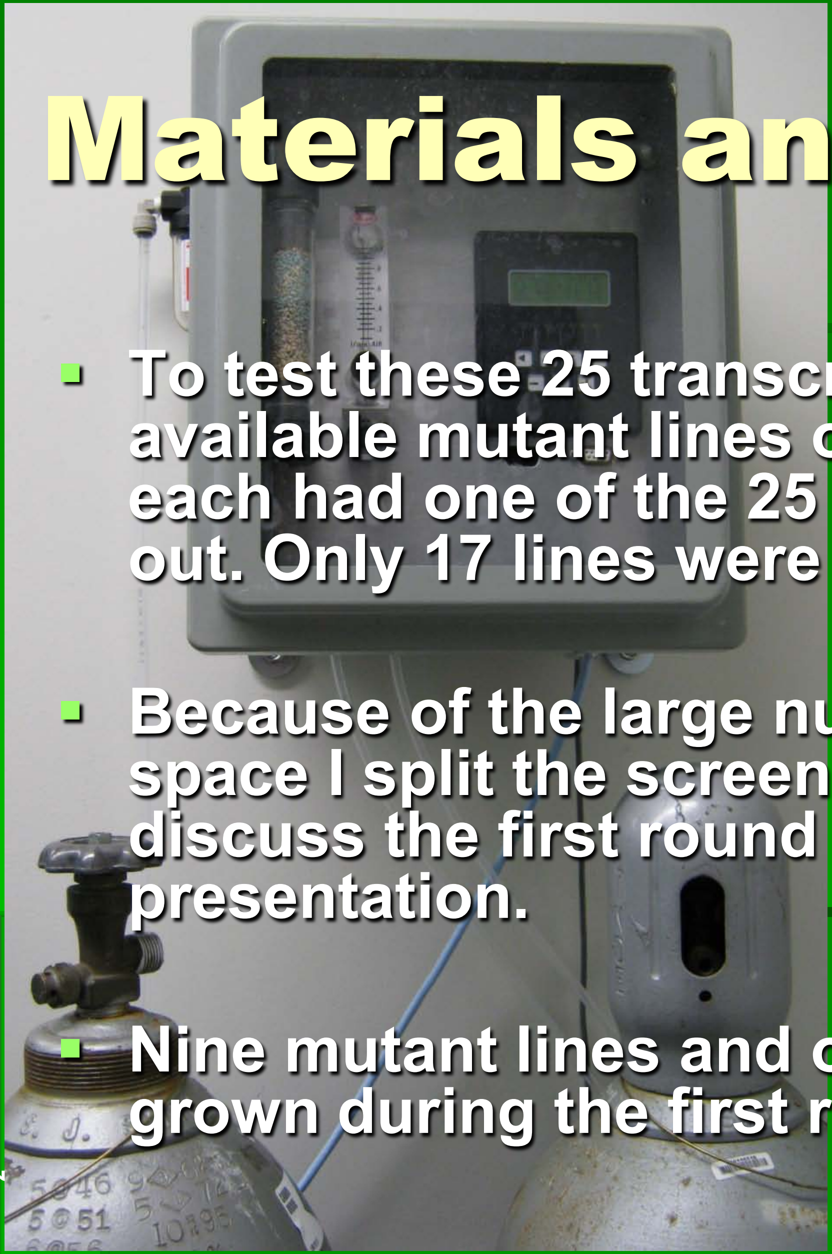
- *Arabidopsis thaliana* is a model genetic plant. Arabidopsis is easily mutated, has a short generation time, is easily grown in a laboratory setting, and has its entire genome sequenced. Not to mention that mutants of *A. thaliana* are available for purchase online from TAIR (The Arabidopsis Information Resource), where you can search for mutants by gene.


Arabidopsis thaliana

Hypothesis

- One or more of these genes is required for the stimulation of metabolism and growth in elevated [CO_2].
- Therefore, the aim is to identify genes that are required for growth stimulated by elevated [CO_2] by identifying knockout plants where growth is not stimulated in the presence of elevated [CO_2].

Materials and Methods



CO_2 Control

- To test these 25 transcription factors I obtained available mutant lines of *Arabidopsis thaliana* who each had one of the 25 genes of interest knocked out. Only 17 lines were available through TAIR.

- Because of the large number of lines and limited space I split the screening in two parts. I will only discuss the first round of the screening during this presentation.

- Nine mutant lines and one wild type line were grown during the first round of screening.

Materials and Methods

Growth Conditions:

Environmental Growth Chamber

- The lines were grown in environmental growth chambers equipped to control [CO_2]. Half of each line was grown in elevated [CO_2] and half in ambient [CO_2].
  - Ambient: 400ppm CO_2
  - Elevated: 1000ppm CO_2
  - Day Length: 9 hours
  - Light: 250 µmol m^{-2} s^{-1}
  - Humidity: 70%
  - Temperature: 21°C Day
18°C Night

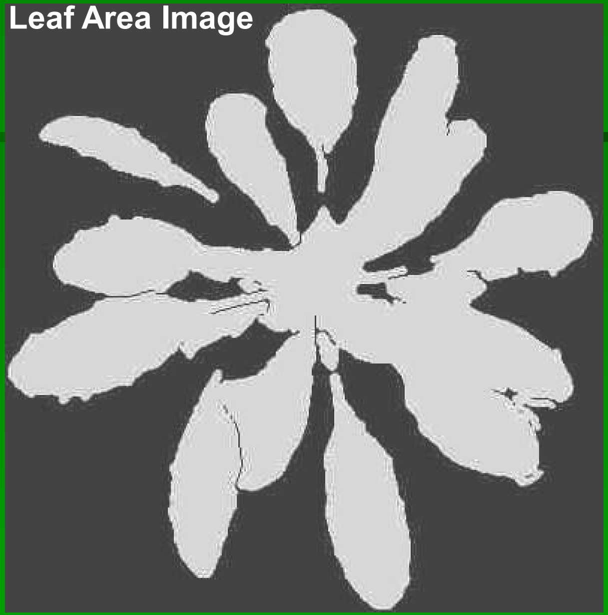
Materials and Methods

Measurements:

- Measurements taken every 1-3 days
  - Digital Pictures
  - Leaf Area
  - Leaf Count
- SPAD measurements were taken twice
- Final Above Ground Biomass

Materials and Methods

Digital Analysis:



- Approximately 3,080 pictures were taken over the course of the growing period.
- Digital pictures allows for visual comparisons.
- The pictures were also processed using Image J software to calculate leaf area as seen above. The program selects all pixels in the picture that are green. Because each picture contained a scale, pixels in the picture were related to a known size and the area of the plant was calculated based on the number of green pixels in the image.

Materials and Methods
SPAD:

- SPAD meters measure the amount of light passing through a leaf at a specific wave length.
- This measure is used a proxy for chlorophyll content.

Materials and Methods
Above Ground:

- At the end of the growing period all above ground tissue was collected and dried in an oven at 70°C.
- After three days of drying samples were removed from the oven and massed.

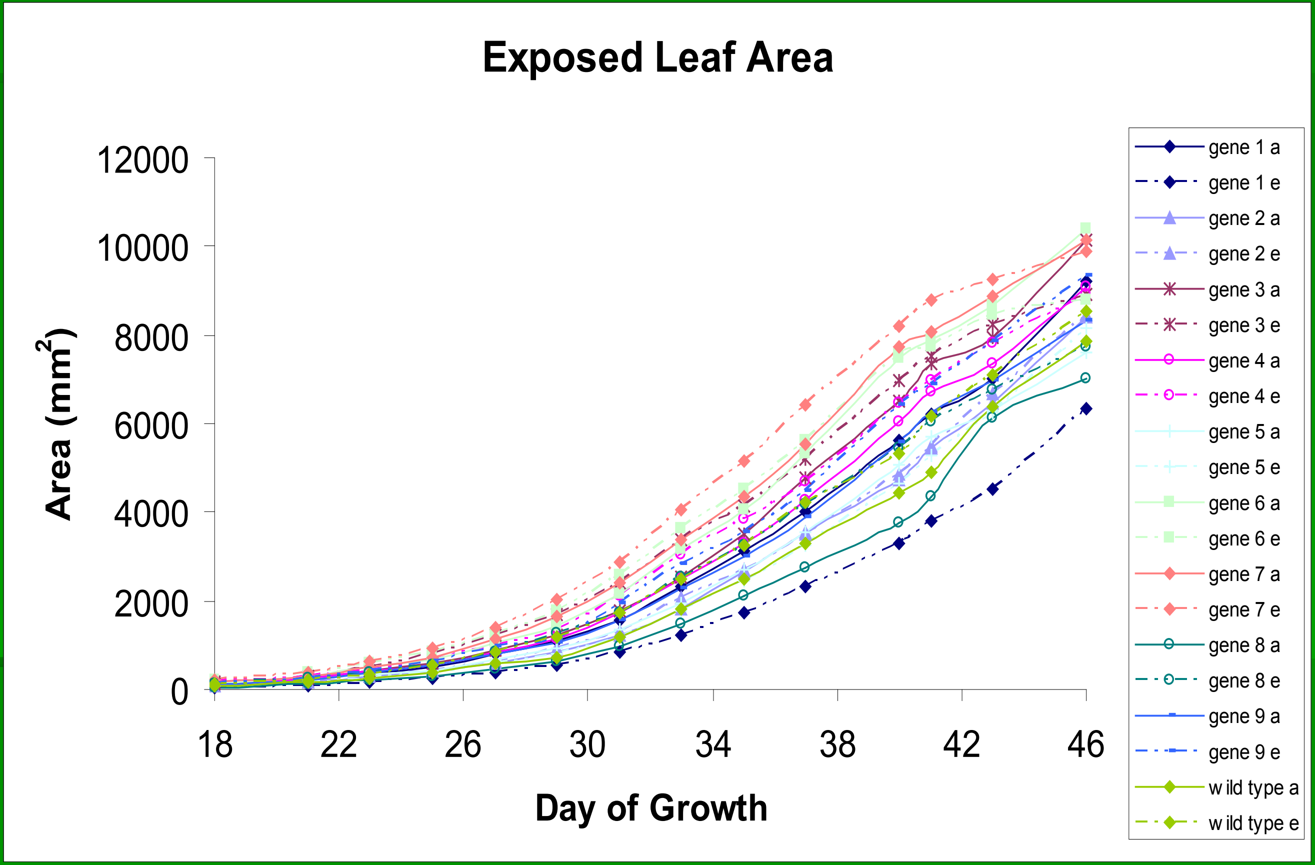
Results

- As expected the Wild Type plants showed a stimulation of growth at elevated CO_2.
- Wild Type plants grown in elevated [CO_2] were larger, had more leaves and larger mass than did their ambient grown counterparts.
- How did the mutant lines compare?

Results:

Leaf Area

- The genotype lacking “Gene 1” and the genotype lacking “Gene 5” failed to utilize elevated [CO_2] to increase leaf area.
- The genotype lacking “Gene 1” appears to have performed better under ambient conditions demonstrated here by larger leaf area throughout the growing period.
- The genotype lacking “Gene 5” showed no difference in leaf area between treatments.



Results:

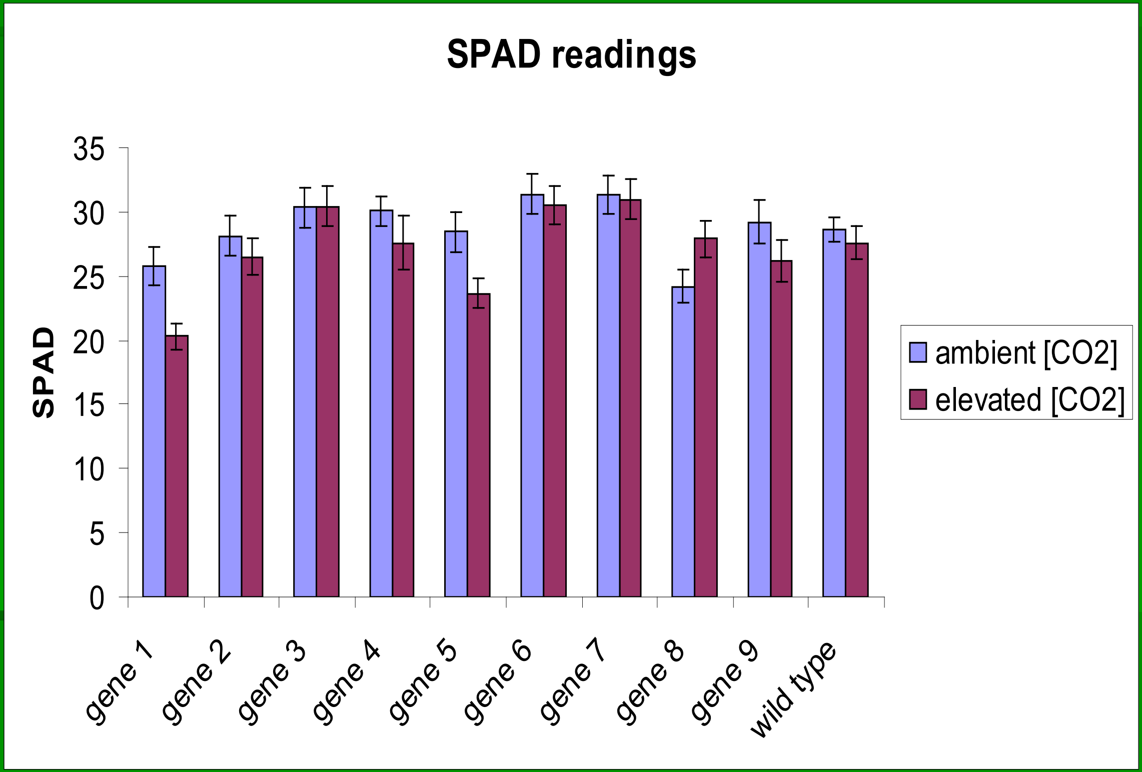
Phenotypes

- The line "Gene 1" showed a visible phenotype separate from size and leaf number, it appeared pale green in comparison to the wild type.
- Because this pale green is possibly due to a low chlorophyll content, SPAD readings were taken to quantify the observed phenotype.
- The pale green color observed in the mutant line was more apparent in the elevated grown plants than in their ambient counterparts consistent with the SPAD data.

Results:

SPAD Readings

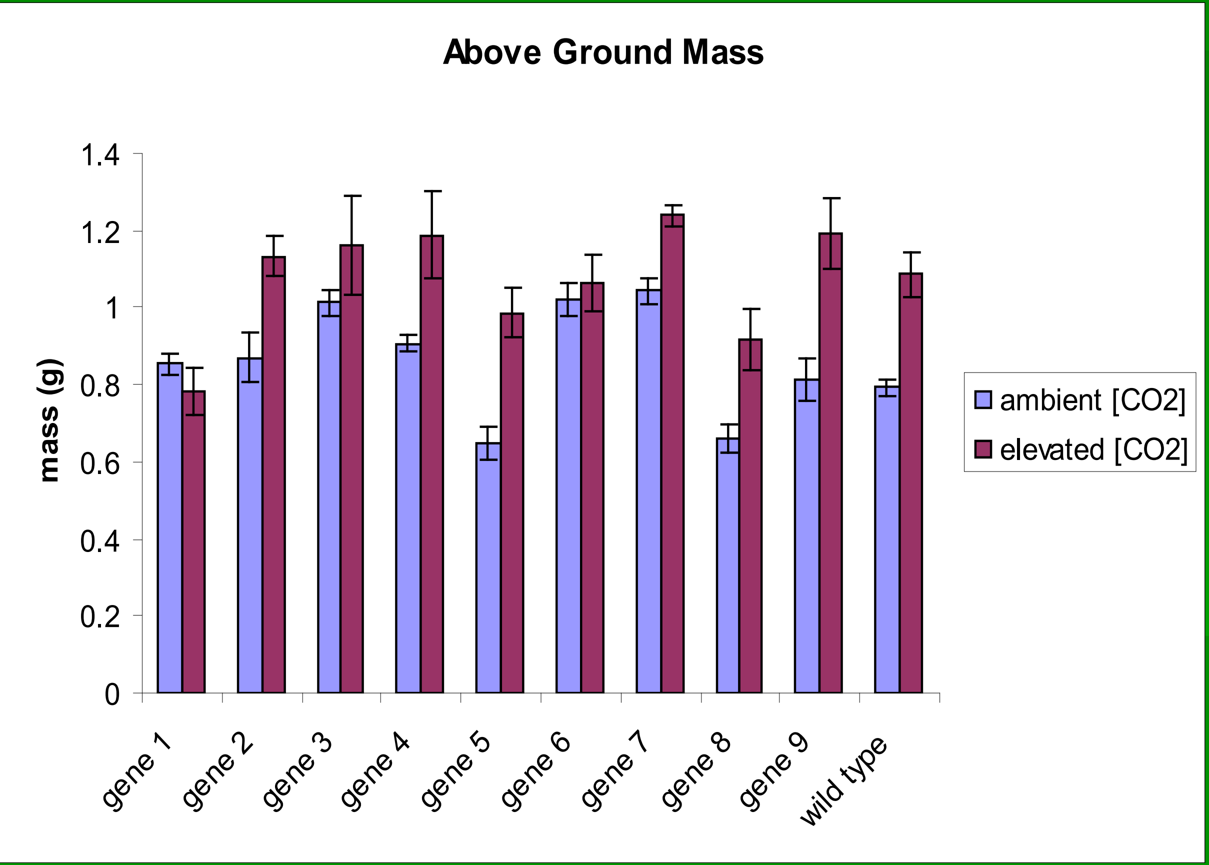
- The genotypes lacking "Gene 1" and "Gene 5" show a clear difference between treatments, a trend not observed in the wild type.
- The genotypes lacking "Gene 1" and "Gene 5" were 21.2% and 20.4% darker in ambient [CO_2] than in elevated [CO_2] respectively.
- This provides a quick phenotyping method in subsequent experiments



Results:

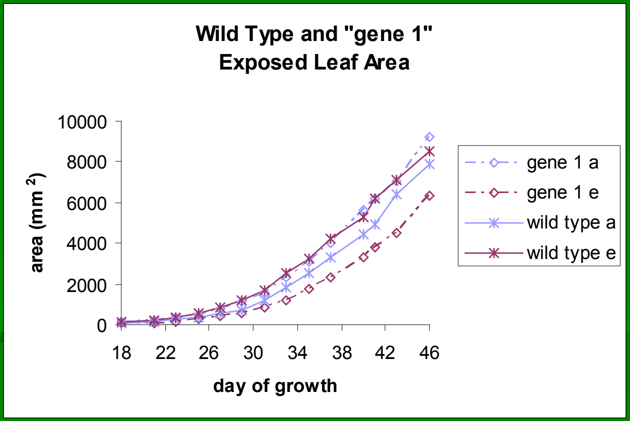
Final Above Ground Mass

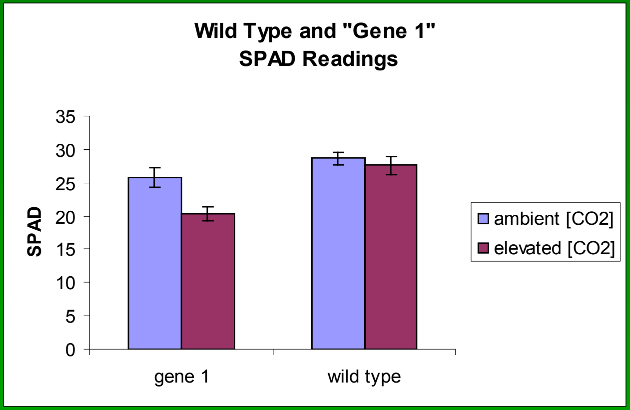
- The genotypes lacking "Gene 1" and "Gene 6" appear to have no stimulation in above ground biomass when exposed to elevated [CO_2].
- Wild type plants showed a 36.9% stimulation in growth under elevated [CO_2].

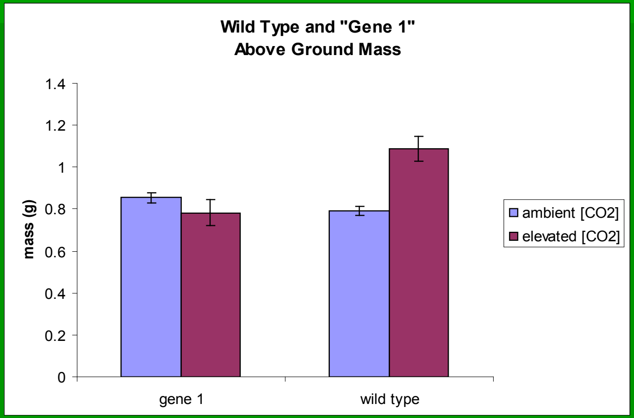


Conclusion

- There are many interesting results from this first round of screening. The four lines lacking "Gene 1", "Gene 3", "Gene 5", and "Gene 6" all failed to utilize elevated [CO_2] in at least one aspect of their growth as predicted by the hypothesis.
- The genotype lacking "Gene 1" did not show the typical CO_2 treatment effect in area, leaf count, or mass when grown under elevated [CO_2] conditions.
- The "Gene 1" line also appeared pale, and showed lower SPAD readings in elevated [CO_2] than in ambient. This is an important feature not only for the implications of reduced chlorophyll count but also because it allows the line to be easily phenotyped in a non-destructive manor.







Conclusion

- My experiment revealed that “Gene 1” is a good candiate for being key to controlling the the stimulation of metabolism and growth in plants grown at elevated [CO_2].

Follow Ups

- I have designed an experiment to further test “Gene 1” that will hopefully help to further elucidate the gene’s function.
- I will grow the mutant line with wild type controls for 30 days and then drop half of each line into elevated [CO_2].
- Over the course of twelve days following the [CO_2] treatment I will take intensive measurements to try and identify any metabolic and physiological changes that may be taking place during acclimation to elevated [CO_2].
- These measurements will include carbohydrate, RNA, leaf area, carbon/nitrogen ratio, SPAD, DNA, and gas exchange measurements to characterize any metabolic and physiological changes that may be taking place.

Thank You!

I had a great summer and would like to thank GCEP for allowing me this opportunity. I appreciate all the hard work that goes into making this program possible and hope to see you all again.

Best wishes!

-Ryan Boyd